UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/806,462	03/22/2002	Koji Kigawa	084335/0134	4495

23533 7590 06/01/2005

STEPHEN B MAEBIUS
FOLEY AND LARDNER
3000 K STREET N W SUITE 500
WASHINGTON, DC 20007-5109

EXAMINER	
STRZELECKA, TERESA E	
ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 06/01/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/806,462	KIGAWA ET AL.	
	Examiner	Art Unit	
	Teresa E. Strzelecka	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 January 2005 and 09 March 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-9,13 and 24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-9,13 and 24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on January 11, 2005 and March 9, 2005 has been entered.

2. Claims 1-9, 13 and 22-24 were previously pending. Applicants cancelled claims 2, 22 and 23 and amended claims 1, 5-8 and 24. Claims 1, 3-9, 13 and 24 are pending and will be examined.

3. Applicants' amendments overcame the following rejections: rejection of claims 1, 2, 4-9 and 22 under 35 U.S.C. 102(b) as anticipated by Kigawa et al.; rejection of claim 3 under 35 U.S.C. 103(a) over Kigawa et al. and Sena et al.; rejection of claim 13 under 35 U.S.C. 103(a) over Kigawa et al. and Kigawa-2 et al.; rejection of claims 23 and 24 under 35 U.S.C. 103(a) over Kigawa et al.

4. This office action presents new grounds for rejection necessitated by claim amendments. Most of Applicants' arguments are moot in view of new grounds for rejection, however, as the rejections are restated under 35 U.S.C. 103(a), the arguments regarding unexpected results are addressed below.

Response to Arguments

5. Applicant's arguments filed March 9, 2005 have been fully considered but they are not persuasive.

In particular, Applicants argue that since Kigawa et al. disclose a range of ATPγS/RecA ratios which overlaps the claimed range of 5/1or less, not specific ratios, the unexpected results obtained by Applicants should be taken into account when comparing Applicants' invention with

prior art. However, results presented by Applicants regarding recombination efficiency of mixtures prepared with different ratios of ATPγS/RecA and ATPγS/nucleotide do not show a clear tendency to increase recombination efficiency under all possible reaction conditions. For example, conditions A and B (Table 1) include ATPγS/RecA ratios of 1/1 and ½, respectively, and ATPγS/nucleotide ratio of 1/5. For these reaction conditions, the recombination efficiencies obtained are 55.7 and 83.6%, respectively, in 100 mM NaCl (Table 2). No zero-salt results are presented. Conditions C and D have the same ATPγS/RecA ratios as conditions A and B, respectively, but their ATPγS/nucleotide ratio is ¼. As can be seen from Table 2, the recombination efficiencies for these two conditions are 80.5 and 87.5%, respectively, at 100 mM NaCl; the value of recombination frequency for condition C only was provided at zero salt concentration. Therefore, as can be seen from these two sets of conditions, increasing ATPγS/nucleotide ratio basically removes the difference in recombination efficiency due to decreased ATPγS/RecA ratio at 100 mM NaCl.

Conditions F-H, which fall within the scope of presently amended claims, have varied ratios of both ATPγS/nucleotide and ATPγS/RecA, therefore, it is not clear whether the observed slight increase in recombination efficiency is due to decrease in ATPγS/nucleotide ratio or to a decrease in ATPγS/RecA ratio, since in all of these samples both ratios decrease.

In view of the above facts presented by Applicants, Applicants' results cannot be considered unexpected for all possible reaction conditions and combinations of ATPγS/nucleotide ratio and ATPγS/RecA ratio.

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 1, 4-9 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa et al. (WO 98/08975; cited in the previous office action), as evidenced by Sena et al. (U.S. Patent No. 5,670,316; cited in the previous office action).

A) Regarding claim 1, Kigawa et al. teach a method of preparing a RecA/single-stranded nucleic acid probe, the method comprising reacting a single-stranded nucleic acid probe sample containing a homologous probe with a RecA recombinase in the presence of ATPγS, wherein the number of ATPγS molecules is one quarter or more than the number of molecules of nucleotide residues in the single-stranded nucleic acid probe and 5 times or less than the number of RecA molecules (Kigawa et al. teach preparing a RecA /single-stranded DNA complex by mixing a RecA recombinase with the homologous nucleic acid probe in the presence of non-hydrolyzable nucleotide cofactor ATPγS (page 15, lines 4-14; page 14, lines 15-28). Kigawa et al. teach that the reaction mix may contain 0.05-5 mM GTPγS, or 0.01-3 mM ATPγS or 0.3-3 mM ATPγS, 0.002-0.025 mM RecA protein, and 0.5-150 ng of homologous probe per reaction (page 17, lines 2-5). Kigawa et al. do not specifically teach that a number of molecules (= molar concentration) of GTPγS or ATPγS is 5-fold or less than the number of molecules of RecA recombinase, however, if a reaction mix contains 0.05 mM GTPγS and 0.025 mM of RecA recombinase, the number of co-factor molecules is twice the number of RecA molecules. If the reaction mix contains 0.01 mM

ATPγS and 0.025 mM of RecA recombinase, the number of co-factor molecules is 0.4 times the number of RecA molecules, etc. Therefore, there are quite a few combinations of reaction conditions under which the number of co-factor molecules is 10-fold or less than the number of molecules of RecA recombinase.

Furthermore, Kigawa et al. teach a specific reaction mixture, No. 23 (Table 1, page 26), which contained 1 ng of 275 bp homologous probe, 500 ng of λ DNA fragments and 15 μg of RecA. The reaction volume was 9 μl, and contained 0.3 mM GTPγS (page 23, lines 7-17). Again, Kigawa et al. do not specifically teach that a number of molecules (= molar concentration) of GTPγS is 5-fold or less than the number of molecules of RecA recombinase and one quarter (= 25%) or more of the number of molecules of nucleotide residue in the nucleic acid probe. However, calculation of the molar concentrations of RecA and nucleic acid probe gives the following values for the molar concentration of these two components: RecA concentration of 0.044 mM (assuming molecular weight 37,842, as evidenced by Sena et al., col. 2, lines 42, 43), and concentration of the homologous 275 bp probe of 0.011 μM (assuming a molecular weight of one nucleotide of 330). Therefore, the molar ratio of GTPγS to RecA is 6.8, which is less than 10-fold, and the molar ratio of GTPγS to nucleotide residue in the nucleic acid probe is 27,000, which is more than 25% of the number of nucleotide residues in the homologous probe.).

Regarding claim 4, Kigawa et al. teach a mixture of homologous and heterologous probes (page 8, lines 16-23; page 16, lines 28-31; page 17, lines 1-5; page 23, lines 7-18).

Regarding claim 5, Kigawa et al. teach magnesium ion concentrations from 1-30 mM (page 16, line 31), or 2 mM (page 23, lines 13-18), anticipating the range of 0.5 to 2 mM.

Regarding claims 6 and 7, Kigawa et al. teach RecA from E. coli (page 9, line 14).

Regarding claim 8, Kigawa et al. teach RecA which has a label (page 15, lines 17, 18).

Regarding claim 9, Kigawa et al. teach a homologous probe which has a label or a ligand (page 12, lines 18-23).

Regarding claim 24, Kigawa et al. teach that the reaction mix may contain 0.05-5 mM GTPγS, or 0.01-3 mM ATPγS or 0.3-3 mM ATPγS, 0.002-0.025 mM RecA protein, and 0.5-150 ng of homologous probe per reaction (page 17, lines 2-5). Kigawa et al. do not specifically teach that a number of molecules (= molar concentration) of GTPγS or ATPγS is 5-fold or less than the number of molecules of RecA recombinase, however, if a reaction mix contains 0.05 mM GTPγS and 0.025 mM of RecA recombinase, the number of co-factor molecules is twice the number of RecA molecules. If the reaction mix contains 0.01 mM ATPγS and 0.025 mM of RecA recombinase, the number of co-factor molecules is 0.4 times the number of RecA molecules, etc. Therefore, there are quite a few combinations of reaction conditions under which the number of co-factor molecules is 10-fold or less than the number of molecules of RecA recombinase.

Furthermore, Kigawa et al. teach a specific reaction mixture, No. 23 (Table 1, page 26), which contained 1 ng of 275 bp homologous probe, 500 ng of λ DNA fragments and 15 μg of RecA. The reaction volume was 9 μl, and contained 0.3 mM GTPγS (page 23, lines 7-17). Again, Kigawa et al. do not specifically teach that a number of molecules (= molar concentration) of GTPγS is 10-fold or less than the number of molecules of RecA recombinase and one quarter (= 25%) or more of the number of molecules of nucleotide residue in the nucleic acid probe. However, calculation of the molar concentrations of RecA and nucleic acid probe gives the following values for the molar concentration of these two components: RecA concentration of 0.044 mM (assuming molecular weight 37,842, as evidenced by Sena et al., col. 2, lines 42, 43), and concentration of the homologous 275 bp probe of 0.011 μM (assuming a molecular weight of one nucleotide of 330). Therefore, the molar ratio of GTPγS to RecA is 6.8, which is less than 10-fold, and the molar ratio

of GTPγS to nucleotide residue in the nucleic acid probe is 27,000, which is more than 25% of the number of nucleotide residues in the homologous probe.

Therefore, Kigawa et al. teach ranges of ATPγS/RecA or GTPγS/RecA ratios which are less than five-fold, and a specific ratio which is 6.8/1.

Further, looking at the results of transformation specificity presented by Kigawa et al. in Table 2 on pages 26 and 27, it is clear that the ratio of co-factor to the nucleotide in the probe and to the RecA, as well as the amount of non-specific DNA present, can all be adjusted to obtain the desired reaction specificity. For example, reactions 14-18 in Table 1 contained decreasing total amounts of the 275 bp probe and the same amount of RecA, 6 μg (the GTPγS/RecA ratio for this reaction would be 17/1), and the reaction specificity increased with decreasing amount of the probe, with reaction 18 having higher specificity than reaction 23 (page 27). Further, reaction 22 of Table 1, which differed from the cited reaction 23 in that it contained half the amount of RecA (the GTPγS/RecA ratio for this reaction would be 13.6/1) and half the amount of non-specific probe had higher reaction specificity than reaction 23.

Thus, an ordinary practitioner would have recognized that the optimizable variables of the concentrations of could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific concentrations of components was other than routine, that the products

resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have adjusted the concentrations of non-hydrolyzable cofactor, recombinase and probe in the reaction mixture, as indicated by Kigawa et al. The motivation to do so would have been to optimize the reaction specificity and efficiency.

8. Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa et al. (WO 98/08975; cited in the previous office action) and Sena et al. (U.S. Patent No. 5,670,316; cited in the previous office action).

A) Teachings of Kigawa et al. are discussed above. Kigawa et al. do not teach a homologous probe consisting of two at least two types of homologous probes that are sufficiently complementary to one another.

B) Sena et al. teach double-stranded probes for homologous recombination, the probes consisting of two sequences containing regions of complementary overlaps with each other, with a degree of complementarity between 70 and 100% (col. 3, lines 39-44; col. 12, lines 29-46), which is the degree of complementarity considered as substantial by Applicants (page 10, lines 35, 36; page 11, lines 1-10), therefore Sena et al. teach probes with substantially complementary overlap.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the double-stranded probes of Sena et al. in the method of Kigawa et al. The motivation to do so, provided by Kigawa et al., would have been that using double-stranded probes produced probe:target DNA complexes stable to deproteinization (col. 3, lines 25-30).

9. Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa et al. (WO 98/08975; cited in the previous office action), as evidenced by Sena et al. (U.S. Patent No.

5,670,316; cited in the previous office action), and Kigawa-2 et al. (EP 0 687 738 A1; cited in the previous office action).

A) Regarding claim 13, Kigawa et al. teach RecA labeled with a label or a ligand, but do not teach biotin or digoxigenin.

B) Regarding claim 13, Kigawa-2 et al. teach RecA protein labeled with biotin or digoxigenin (col. 10, lines 1-15).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used labeled RecA protein of Kigawa-2 et al. in the method of Kigawa et al. The motivation to do so, provided by Kigawa-2 et al., would have been that labeling the protein provided a sensitive and simple method of detecting hybridization complexes (col. 4, lines 3-11).

10. No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Teresa E. Strzelecka whose telephone number is (571) 272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

May 24, 2005

TERESA STRZELECKA
PATENT EXAMINER
Teresa Strzelecka